PRODUCTION OF GP88 BY TUMORIGENIC AND NON-TUMORIGENIC CELLS

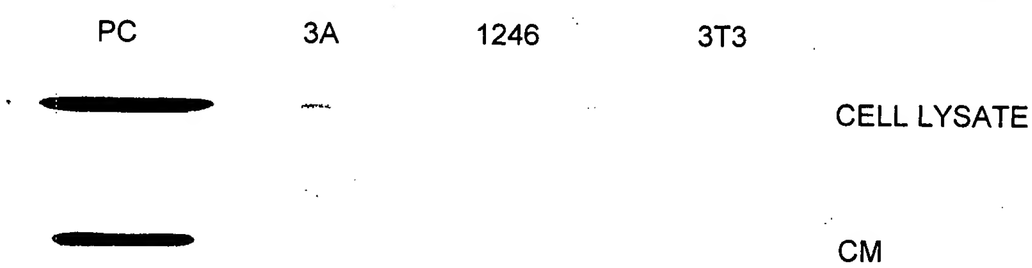


FIG.1A

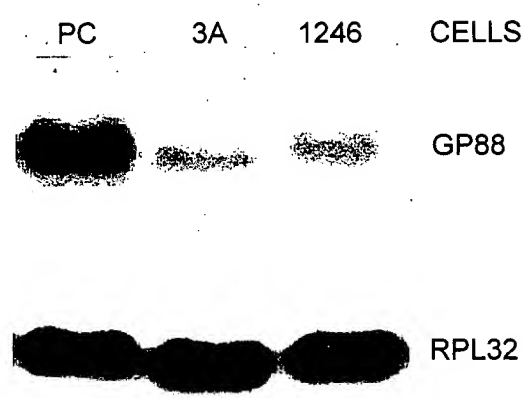


FIG.1B

GP88 mRNA EXPRESSION IN VARIOUS CULTURE CONDITIONS

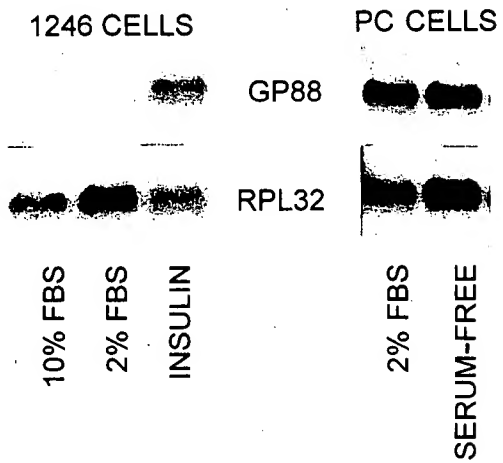


FIG.1C

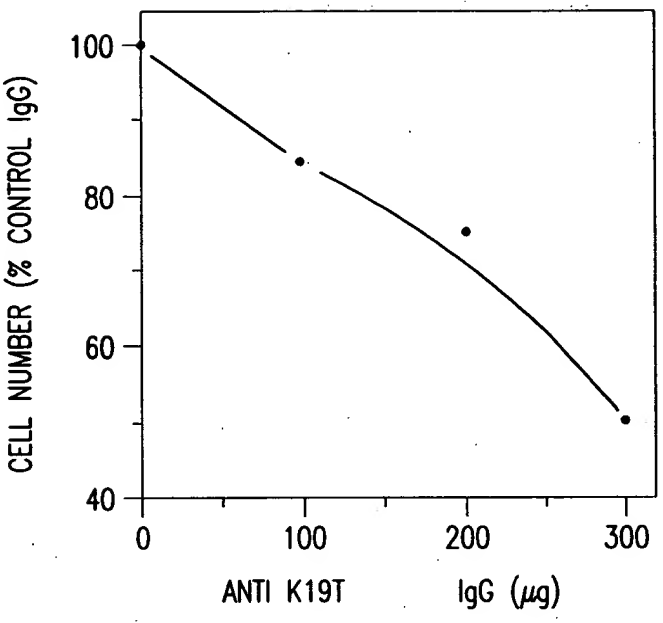

100
80
60
40
CELL NUMBER (% CONTROL IgG)
0
100
200
300
ANTI K19T
IgG (μg)

FIG.2

ABSENCE OF TUMOR FORMATION IN C3H MICE BY INHIBITION OF GP88 EXPRESSION

GP88 ANTISENSE TRANSFECTED PC CELLS

CONTROL TRANSFECTED PC CELLS

FIG.3

GP88 PROTEIN EXPRESSION IN TUMOR AND SURROUNDING TISSUES

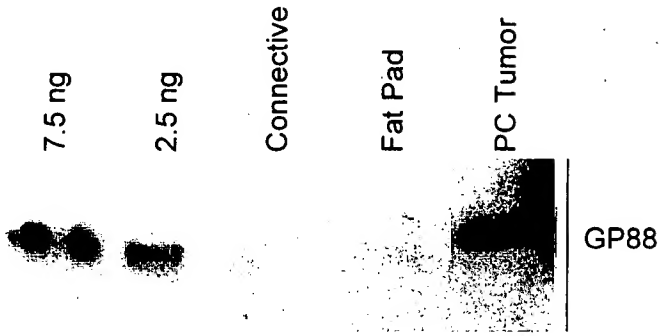


FIG.4

GP88 mRNA EXPRESSION IN ESTROGEN-DEPENDENT AND INDEPENDENT HUMAN MAMMARY CARCINOMA CELLS

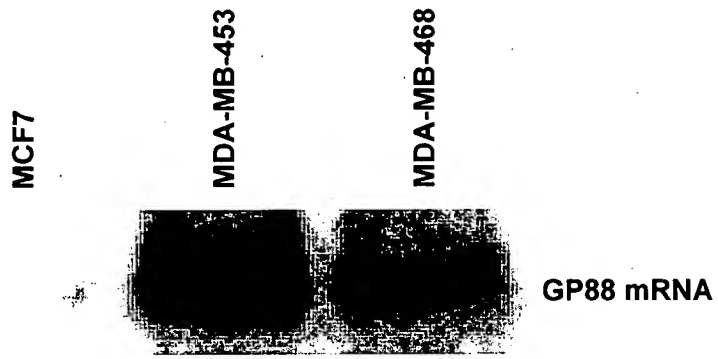


FIG.5

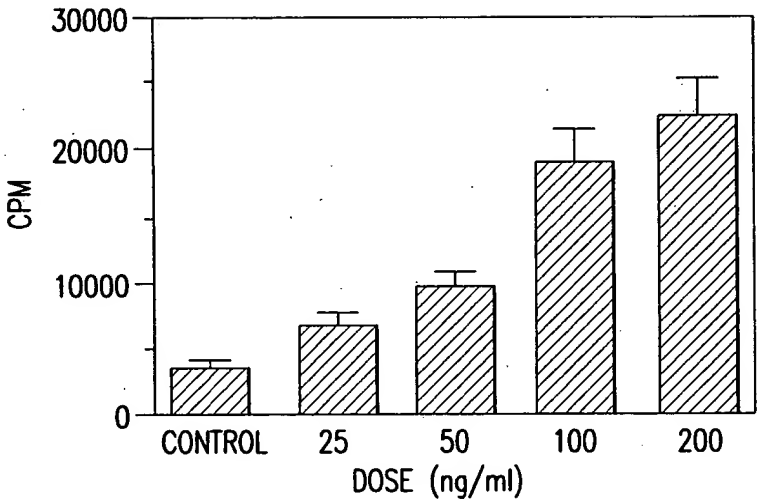

30000
20000
10000
0
CPM
CONTROL
25
50
100
200
DOSE (ng/ml)

FIG.6A

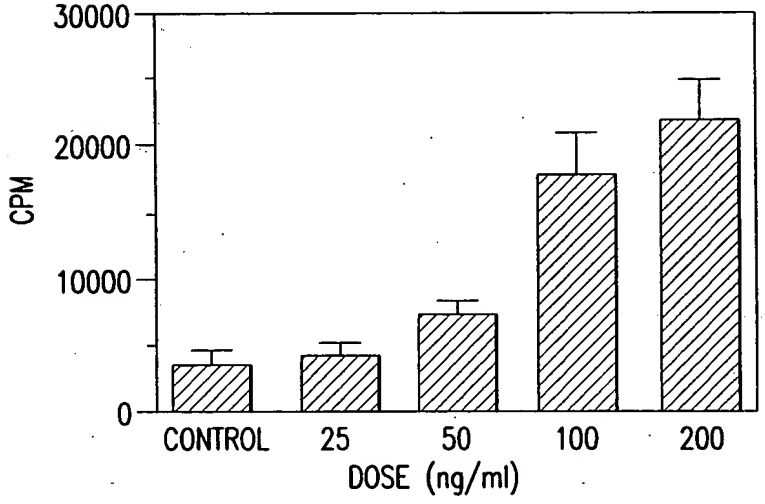

30000
20000
10000
0
CPM
CONTROL
25
50
100
200
DOSE (ng/ml)

FIG.6B

EXPRESSION OF GP88 IN ANTISENSE AND CONTROL TRANSFECTED PC CELLS

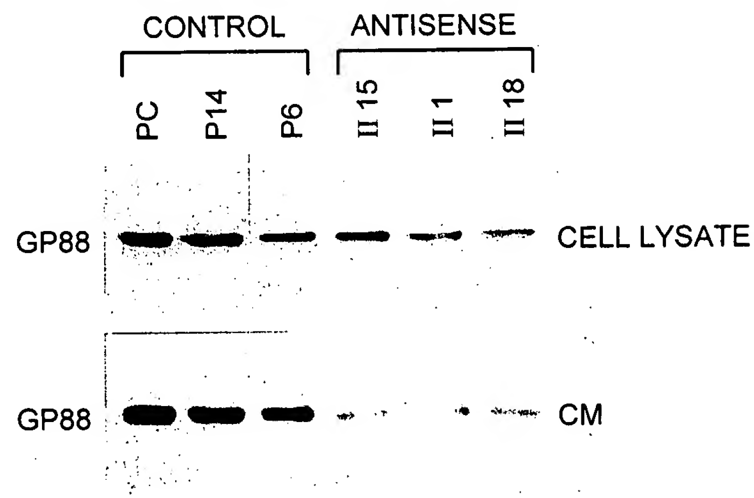


FIG.7

Mouse GP88 cDNA

```
C GGA CCC CGA CGC AGA CAG ACC ATG TGG GTC CTG ATG AGC TGG CTG     46
                              M   W   V   L   M   S   W   L       8

GCC TTC GCG GCA GGG CTG GTA GCC GGA ACA CAG TGT CCA GAT GGG CAG     94
A   F   A   A   G   L   V   A   G   T   Q   C   P   D   G   Q     24

TTC TGC CCT GTT GCC TGC TGC CTT GAC CAG GGA GGA GCC AAC TAC AGC     142
F   C   P   V   A   C   C   L   D   Q   G   G   A   N   Y   S     40

TGC TGT AAC CCT CTT CTG GAC ACA TGG CCT AGA ATA ACG AGC CAT CAT     190
C   C   N   P   L   L   D   T   W   P   R   I   T   S   H   H     56

CTA GAT GGC TCC TGC CAG ACC CAT GGC CAC TGT CCT GCT GGC TAT TCT     238
L   D   G   S   C   Q   T   H   G   H   C   P   A   G   Y   S     72

TGT CTT CTC ACT GTG TCT GGG ACT TCC AGC TGC TGC CCG TTC TCT AAG     286
C   L   L   T   V   S   G   T   S   S   C   C   P   F   S   K     88

GGT GTG TCT TGT GGT GAT GGC TAC CAC TGC TGC CCC CAG GGC TTC CAC     334
G   V   S   C   G   D   G   Y   H   C   C   P   Q   G   F   H     104

TGT AGT GCA GAT GGG AAA TCC TGC TTC CAG ATG TCA GAT AAC CCC TTG     382
C   S   A   D   G   K   S   C   F   Q   M   S   D   N   P   L     120

GGT GCT GTC CAG TGT CCT GGG AGC CAG TTT GAA TGT CCT GAC TCT GCC     430
G   A   V   Q   C   P   G   S   Q   F   E   C   P   D   S   A     136

ACC TGC TGC ATT ATG GTT GAT GGT TCG TGG GGA TGT TGT CCC ATG CCC     478
T   C   C   I   M   V   D   G   S   W   G   C   C   P   M   P     152

CAG GCC TCT TGC TGT GAA GAC AGA GTG CAT TGC TGT CCC CAT GGG GCC     526
Q   A   S   C   C   E   D   R   V   H   C   C   P   H   G   A     168

TCC TGT GAC CTG GTT CAC ACA CGA TGC GTT TCA CCC ACG GGC ACC CAC     574
S   C   D   L   V   H   T   R   C   V   S   P   T   G   T   H     184

ACC CTA CTA AAG AAG TTC CCT GCA CAA AAG ACC AAC AGG GCA GTG TCT     622
T   L   L   K   K   F   P   A   Q   K   T   N   R   A   V   S     200

TTG CCT TTT TCT GTC GTG TGC CCT GAT GCT AAG ACC CAG TGT CCC GAT     670
L   P   F   S   V   V   C   P   D   A   K   T   Q   C   P   D     216
```

FIG.8A

Mouse GP88 cDNA (continued)

```
GAT TCT ACC TGC TGT GAG CTA CCC ACT GGG AAG TAT GGC TGC TGT CCA   718
D   S   T   C   C   E   L   P   T   G   K   Y   G   C   C   P     232

ATG CCC AAT GCC ATC TGC TGT TCC GAC CAC CTG CAC TGC TGC CCC CAG   766
M   P   N   A   I   C   C   S   D   H   L   H   C   C   P   Q     248

GAC ACT GTA TGT GAC CTG ATC CAG AGT AAG TGC CTA TCC AAG AAC TAC   814
D   T   V   C   D   L   I   Q   S   K   C   L   S   K   N   Y     264

ACC ACG GAT CTC CTG ACC AAG CTG CCT GGA TAC CCA GTG AAG GAG GTG   862
T   T   D   L   L   T   K   L   P   G   Y   P   V   K   E   V     280

AAG TGC GAC ATG GAG GTG AGC TGC CCT GAA GGA TAT ACC TGC TGC CGC   910
K   C   D   M   E   V   S   C   P   E   G   Y   T   C   C   R     296

CTC AAC ACT GGG GCC TGG GGC TGC TGT CCA TTT GCC AAG GCC GTG TGT   958
L   N   T   G   A   W   G   C   C   P   F   A   K   A   V   C     312

TGT GAG GAT CAC ATT CAT TGC TGC CCG GCA GGG TTT CAG TGT CAC ACA  1006
C   E   D   H   I   H   C   C   P   A   G   F   Q   C   H   T     328

GAG AAA GGA ACC TGC GAA ATG GGT ATC CTC CAA GTA CCC TGG ATG AAG  1054
E   K   G   T   C   E   X   G   I   L   Q   V   P   W   M   K     344

AAG GTC ATA GCC CCC CTC CGC CTG CCA GAC CCA CAG ATC TTG AAG AGT  1102
K   V   I   A   P   L   R   L   P   D   P   Q   I   L   K   S     360

GAT ACA CCT TGT GAT GAC TTC ACT AGG TGT CCT ACA AAC AAT ACC TGC  1150
D   T   P   C   D   D   F   T   R   C   P   T   N   N   T   C     376

TGC AAA CTC AAT TCT GGG GAC TGG GGC TGC TGT CCC ATC CCA GAG GCT  1198
C   K   L   N   S   G   D   W   G   C   C   P   I   P   E   A     392

GTC TGC TGC TCA GAC AAC CAG CAT TGC TGC CCT CAG GGC TTC ACA TGT  1246
V   C   C   S   D   N   Q   H   C   C   P   Q   G   F   T   C     408

CTG GCT CAG GGG TAC TGT CAG AAG GGA GAC ACA ATG GTG GCT GGC CTG  1294
L   A   Q   G   Y   C   Q   K   G   D   T   M   V   A   G   L     424

GAG AAG ATA CCT GCC CGC CAG ACA ACC CCG CTC CAA ATT GGA GAT ATC  1342
E   K   I   P   A   R   Q   T   T   P   L   Q   I   G   D   I     440
```

FIG.8B

Mouse GP88 cDNA (continued)

```
GGT TGT GAC CAG CAT ACC AGC TGC CCA GTA GGG CAA ACC TGC TGC CCA   1390
G   C   D   Q   H   T   S   C   P   V   G   Q   T   C   C   P     456

AGC CTC AAG GGA AGT TGG GCC TGC TGC CAG CTG CCC CAT GCT GTG TGC   1438
S   L   K   G   S   W   A   C   C   Q   L   P   H   A   V   C     472

TGT GAG GAC CGG CAG CAC TGT TGC CCG GCC GGG TAC ACC TGC AAC GTG   1486
C   E   D   R   Q   H   C   C   P   A   G   Y   T   C   N   V     488

AAG GCG AGG ACC TGT GAG AAG GAT GTC GAT TTT ATC CAG CCT CCC GTG   1534
K   A   R   T   C   E   K   D   V   D   F   I   Q   P   P   V     504

CTC CTG ACC CTC GGC CCT AAG GTT GGG AAT GTG GAG TGT GGA GAA GGG   1582
L   L   T   L   G   P   K   V   G   N   V   E   C   G   E   G     520

CAT TTC TGC CAT GAT AAC CAG ACC TGT TGT AAA GAC AGT GCA GGA GTC   1630
H   F   C   H   D   N   Q   T   C   C   K   D   S   A   G   V     536

TGG GCC TGC TGT CCC TAC CTA AAG GGT GTC TGC TGT AGA GAT GGA CGT   1678
W   A   C   C   P   Y   L   K   G   V   C   C   R   D   G   R     552

CAC TGT TGC CCC GGT GGC TTC CAC TGT TCA GCC AGG GGA ACC AAG TGT   1726
H   C   C   P   G   G   F   H   C   S   A   R   G   T   K   C     568

TTG CGA AAG AAG ATT CCT CGC TGG GAC ATG TTT TTG AGG GAT CCG GTC   1774
L   R   K   K   I   P   R   W   D   M   F   L   R   D   P   V     584

CCA ACA CCG CTA CTG TAA GGA AGG GCT ACA GAC TTA AGG AAC TCC ACA   1822
P   R   P   L   L   *                                             589

GTC CTG GGA ACC CTG TTC CGA GGG TAC CCA CTA CTC AGG CCT CCC TAG   1870
CGC CTC CTC CCC TAA CGT CTC CCC GGC CTA CTC ATC CTG AGT CAC CCT   1918
ATC ACC ATG GGA GGT GGA GCC TCA AAC TAA AAC CTT CTT TTA TGG AAA   1966
GAA GGC TGT GGC CAA AAG CCC CGT ATC AAA CTG CCA TTT CTT CCG GTT   2014
TCT GTG GAC CTT GTG GCC AGG TGC TCT TCC CGA GCC ACA GGT GTT CTG   2062
TGA GCT TGC TTG TGT GTG TGT GCG CGT GTG CGT GTG TTG CTC CAA TAA   2110
AGT TTG TAC GCT TTC TGA AAA AAA AAA                              2137
```

FIG.8C

Nucleotide sequence of human granulin/epithelin precursor (human GP88).
Human Granulin Genbank M75161$

cgcaggcaga ccatgtggac cttggtgagc tgggtggcct taacagcagg gctggtggct
ggaacgcggt gcccagatgg tcagttctgc cctgtggcct gctgcctgga ccccggagga
gccagctaca gctgctgccg tccccttctg gacaaatggc ccacaacact gagcaggcat
ctgggtggcc cctgccaggt tgatgcccac tgctctgccg gccactcctg catctttacc
gtctcaggga cttccagttg ctgccccttc ccagaggccg tggcatgcgg ggatggccat
cactgctgcc cacggggctt ccactgcagt gcagacggga gatcctgctt ccaaagatca
ggtaacaact ccgtgggtgc catccagtgc cctgatagtc agttcgaatg cccggacttc
tccacgtgct gtgttatggt cgatggctcc tgggggtgct gccccatgcc ccaggcttcc
tgctgtgaag acagggtgca ctgctgtccg cacggtgcct tctgcgacct ggttcacacc
cgctgcatca cacccacggg cacccacccc ctggcaaaga agctccctgc ccagaggact
aacagggcag tggccttgtc cagctcggtc atgtgtccgg acgcacggtc ccggtgccct
gatggttcta cctgctgtga gctgcccagt gggaagtatg gctgctgccc aatgcccaac
gccacctgct gctccgatca cctgcactgc tgcccccaag acactgtgtg tgacctgatc
cagagtaagt gcctctccaa ggagaacgct accacggacc tcctcactaa gctgcctgcg
cacacagtgg gcgatgtgaa atgtgacatg gaggtgagct gcccagatgg ctatacctgc
tgccgtctac agtcgggggc ctggggctgc tgccctttta cccaggctgt gtgctgtgag
gaccacatac actgctgtcc cgcggggttt acgtgtgaca cgcagaaggg tacctgtgaa
caggggcccc accaggtgcc ctggatggag aaggccccag ctcacctcag cctgccagac
ccacaagcct tgaagagaga tgtcccctgt gataatgtca gcagctgtcc ctcctccgat
acctgctgcc aactcacgtc tggggagtgg ggctgctgtc caatcccaga ggctgtctgc
tgctcggacc accagcactg ctgcccccag cgatacacgt gtgtagctga ggggcagtgt
cagcgaggaa gcgagatcgt ggctggactg gagaagatgc ctgcccgccg cggttcctta
tcccacccca gagacatcgg ctgtgaccag cacaccagct gcccggtggg cggaacctgc
tgcccgagcc agggtgggag ctgggcctgc tgccagttgc cccatgctgt gtgctgcgag
gatcgccagc actgctgccc ggctggctac acctgcaacg tgaaggctcg atcctgcgag
aaggaagtgg tctctgccca gcctgccacc ttcctggccc gtagccctca cgtgggtgtg
aaggacgtgg agtgtgggga aggacacttc tgccatgata accagacctg ctgccgagac
aaccgacagg gctgggcctg ctgtccctac gcccagggcg tctgttgtgc tgatcggcgc
cactgctgtc ctgctggctt ccgctgcgca cgcaggggta ccaagtgttt gcgcagggag
gccccgcgct gggacgcccc tttgagggac ccagccttga gacagctgct gtgagggaca
gtactgaaga ctctgcagcc ctcgggaccc cactcggagg gtgccctctg ctcaggcctc
gtactgaaga ctctgcagcc ctcgggaccc cactcggagg gtgccctctg ctcaggcctc
cctagcacct ccccctaacc aaattctccc tggaccccat tctgagctcc ccatcaccat
gggaggtggg gcctcaatct aaggcccttc cctgtcagaa gggggttgag gcaaaagccc
attacaagct gccatcccct ccccgtttca gtggaccctg tggccaggtg cttttcccta
tccacagggg tgtttgtgtg ttgggtgtgc tttcaataaa gtttgtcact ttctt*

FIG.9A

Amino-acid sequence of human granulin/epithelin precursor (human GP88).

MWTLVSWVALTAGLVAGTRCPDGQFCPVACCLDPGGASYSCCRP
LLDKWPTTLSRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPEAVACGDGHHCCPRG
FHCSADGRSCFQRSGNNSVGAIQCPDSQFECPDFSTCCVMVDGSWGCCPMPQASCCED
RVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPAQRTNRAVALSSSVMCPDARSRCPDG
STCCELPSGKYGCCPMPNATCCSDHLHCCPQDTVCDLIQSKCLSKENATTDLLTYLPA
HTVGDVKCDMEVSCPDGYTCCRLQSGAWGCCPFTQAVCCEDHIHCCPAGFTCDTQKGT
CEQGPHQVPWMEKAPAHLSLPDPQALKRDVPCDNVSSCPSSDTCCQLTSGEWGCCPIP
EAVCCSDHQHCCPQRYTCVAEGQCQRGSEIVAGLEKMPARRGSLSHPRDIGCDQHTSC
PVGGTCCPSQGGSWACCQLPHAVCCEDRQHCCPAGYTCNVKARSCEKEVVSAQPATFL
ARSPHVGVKDVECGEGHFCHDNQTCCRDNRQGWACCPYAQGVCCADRRHCCPAGFRCA
RRGTKCLRREAPRWDAPLRDPALRQLL*

FIG.9B

se GP88 protein sequence

```
WVLMSWLAFAAGLVAG   17

  TQCPDGQF-CPVA--CCLDQG-GANYSCCNPLLDTWPRITSHHL    57
DGSC-QTHGHCPAGY-SCLLTVSGTS-SCCPFSKGVSCGDGYHCCPQGFHCSADGKSCFQMSDNPL    120                          g
GAVQCPGSQFECPDSATCCIMVD-G-SWGCCPMPQASCCEDRVHCCPHGASCDLVHTRCVSPTGTHTLLKKFPAQKTNAAVSLPFS    204      f
  VVCPDAKTQCPDDSTCCELP-TGK-YGCCPMPNAICCSDHLHCCPQDTVCDLIQSKCLSKNYTTDLLTKLPGYPVK    278            2,B
EVKC-DMEVSCPEGYTCCALN-TGA-WGCCPFAKAVCCEDHIHCCPAGFOCHTEKGICEMGILQVPWMKKVIAPRRLPDPQILKS    360    1,A
DTPCDDFTR-CPTNNTCCKLN-SGD-WGCCPIPEAVCCSDNQHCCPQGFTCLAQGY-CQKGDTMVAGLEKIPARQTTPLQIG    438        C
DIGCDuHT-SCPVGOTCCPSLK-G-SWACCQLPHAVCCEDRQHCCPAGYTCNVKARTCEKDVDFIQPPVLLTLGPKVG    513           D
NVECGEGHF-CHDNQTCCKDSA-GV-WACCPYLKGVCCRDGRHCCPGGFHCSARGTKCLAKKIPRWDMFLADPVPRPLL    589           e
```

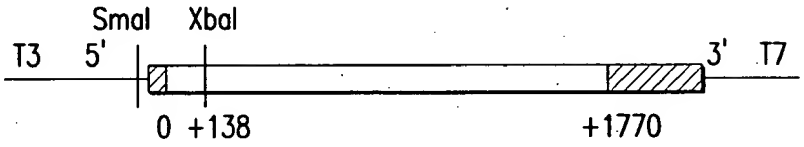
onsensus sequence:

```
  C......C.....CC.....G....CC......CC.D..HCCP....C......C
```

,2:mouse epithelin 1,2.
,B,C,D,e,f,g: granulin A,B,C,D,E,F,G;N-terminus of granulin A,B,C,D have been sequenced.
ouse epithelin precursor sequence is from Plowman et al.(1992).

FIG.10

GP88 cDNA CLONE in SK



STRUCTURE OF $pCMV_4$ EXPRESSION VECTOR

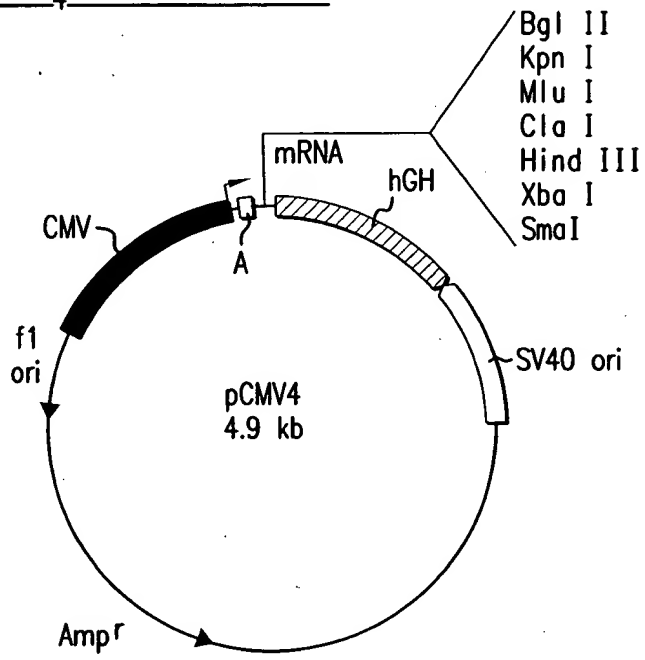


FIG.11

CROSS-LINKING OF ^{125}I-rGP88 TO CCL64 CELLS

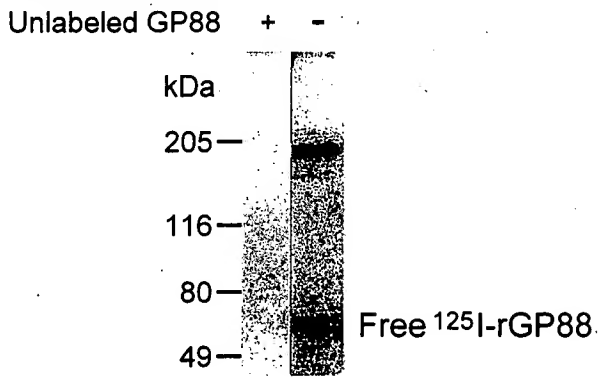


FIG.12

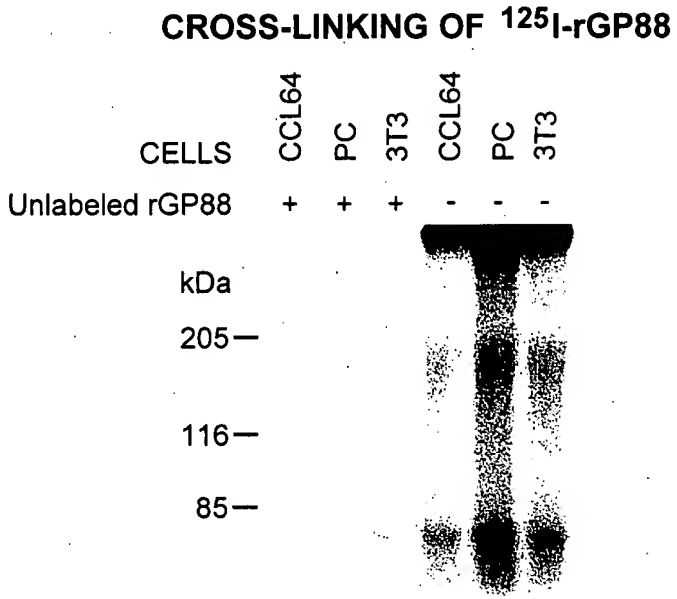


FIG.13

GP88 EXPRESSION IN NON TUMORIGENIC (MCF 10A) AND MALIGNANT (MCF 7, MDA-468) HUMAN MAMMARY EPITHELIAL CELLS

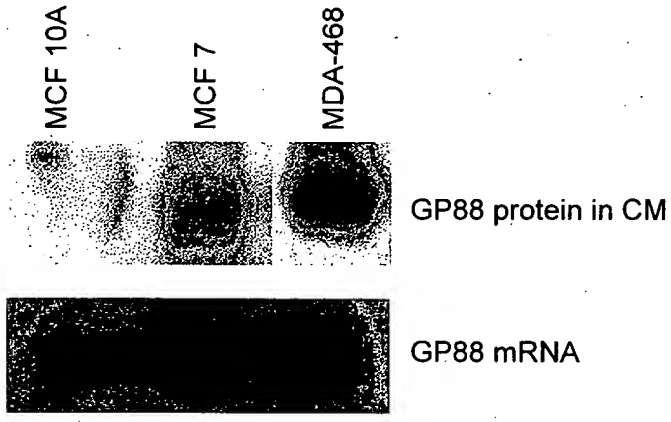


FIG.14

GP88 EXPRESSION IS INHIBITED BY ANTISENSE GP88 cDNA TRANSFECTION IN HUMAN BREAST CARCINOMA MDA-468

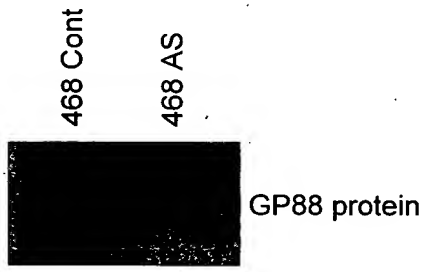


FIG.15

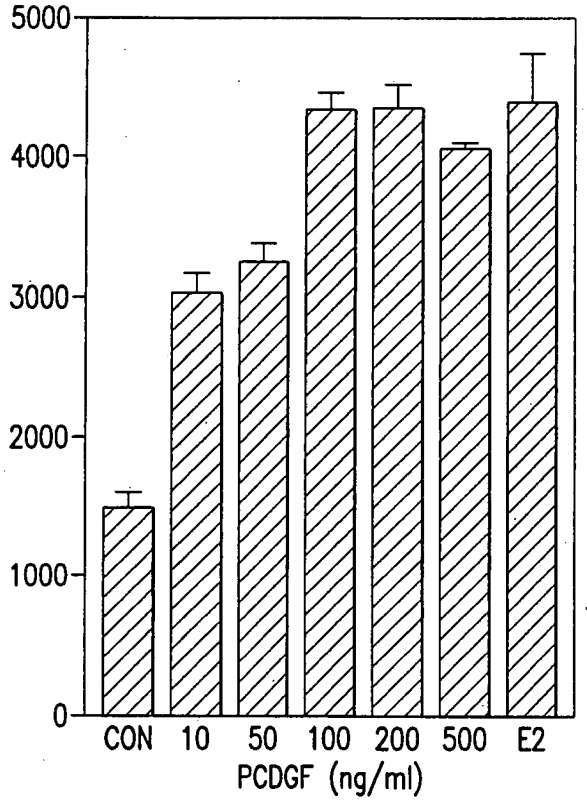


FIG.16A

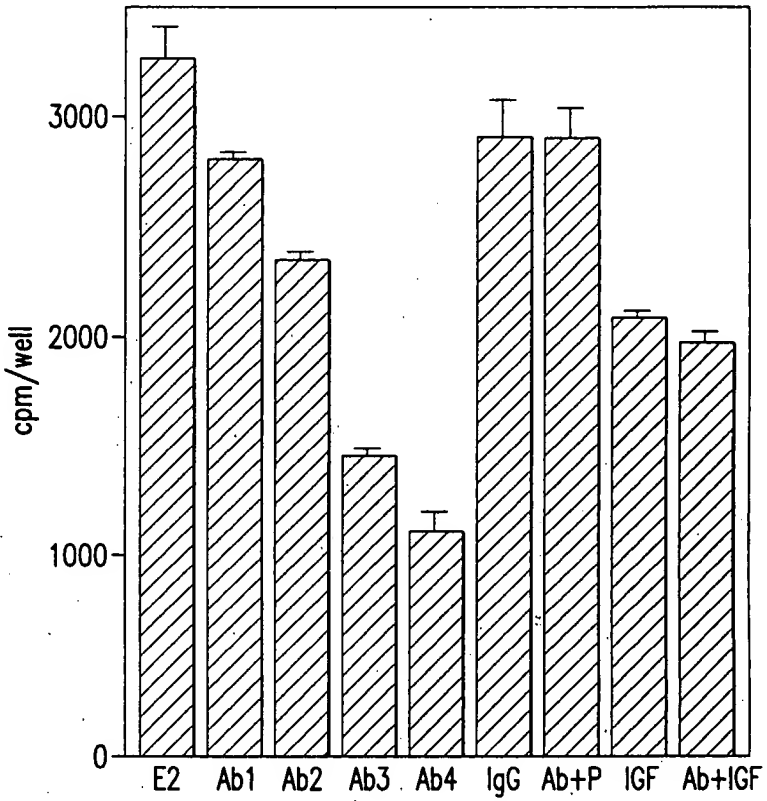


FIG.16B

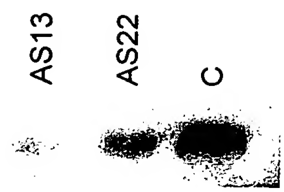


FIG.17A

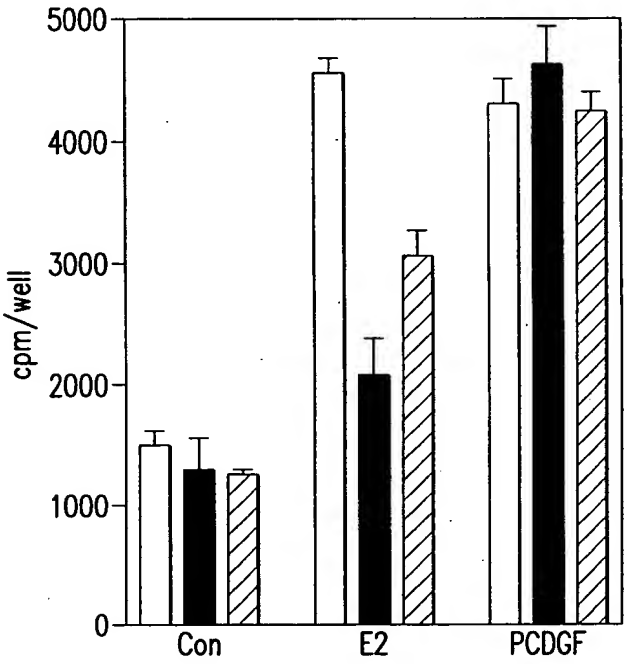


FIG.17B

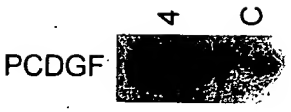


FIG.18A

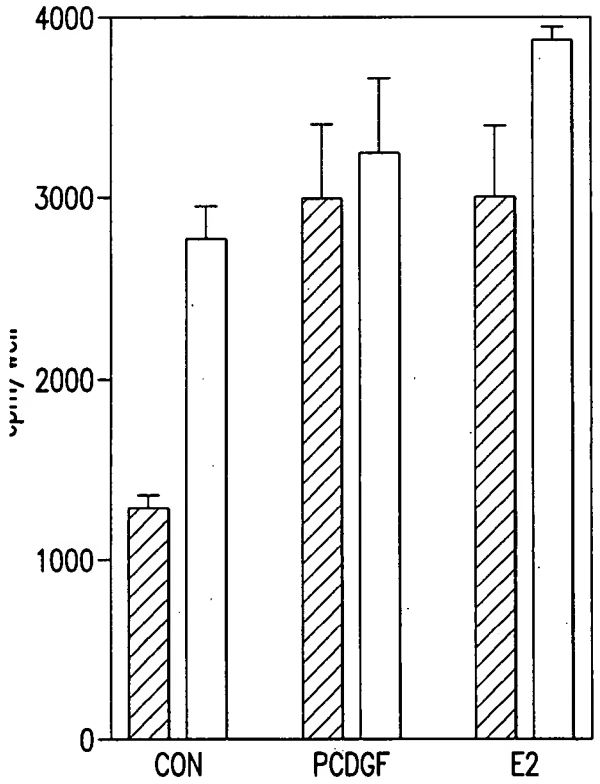


FIG.18B

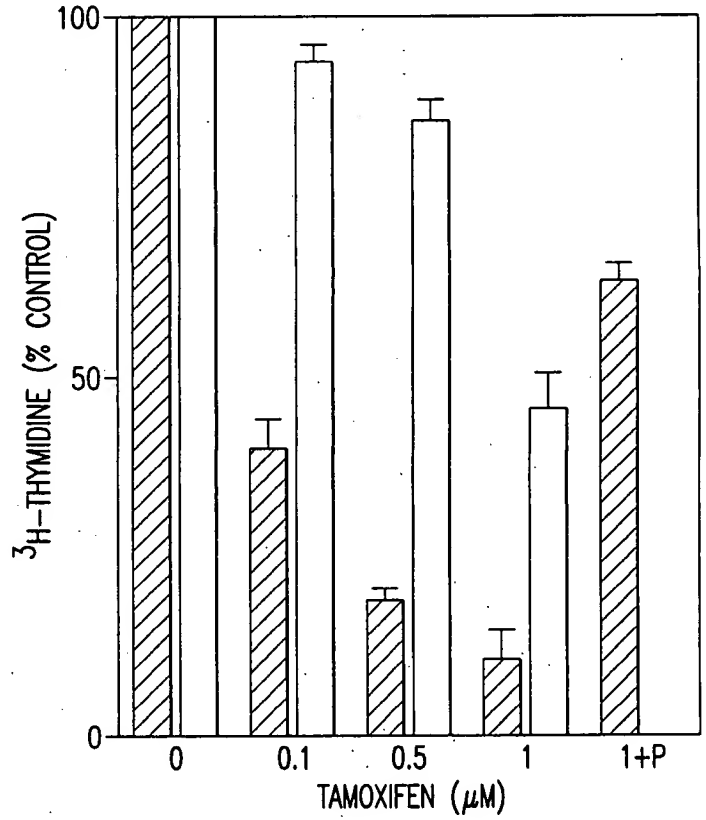


FIG.18C

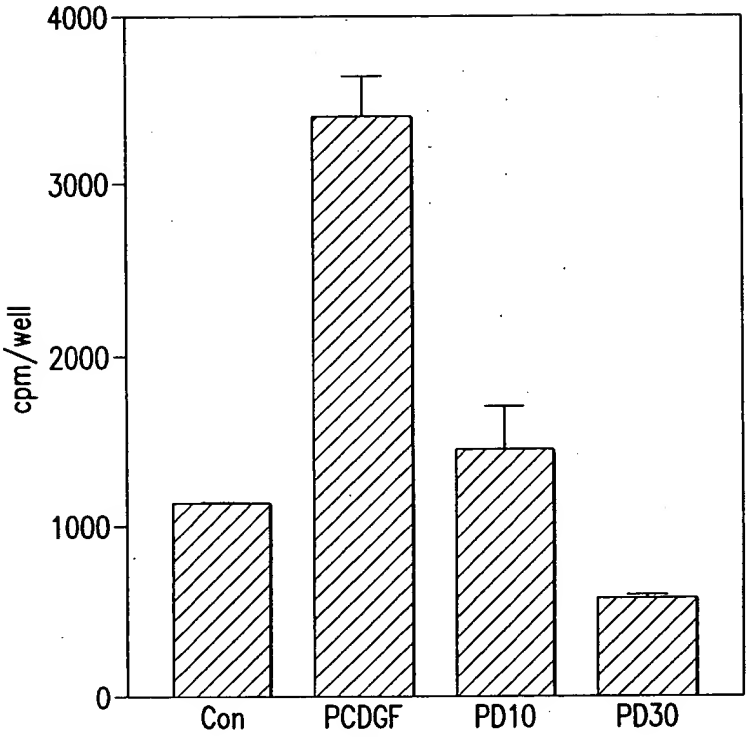


FIG.19A

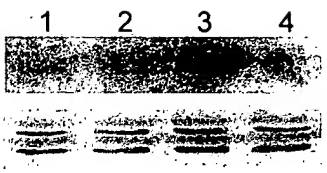


FIG.19B

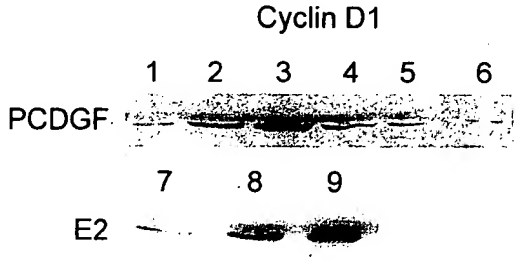


FIG.20A

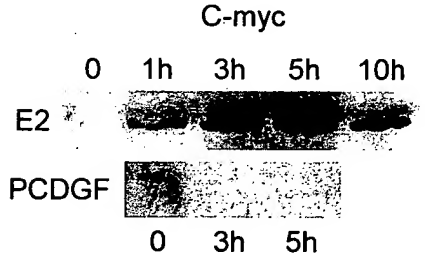


FIG.20B

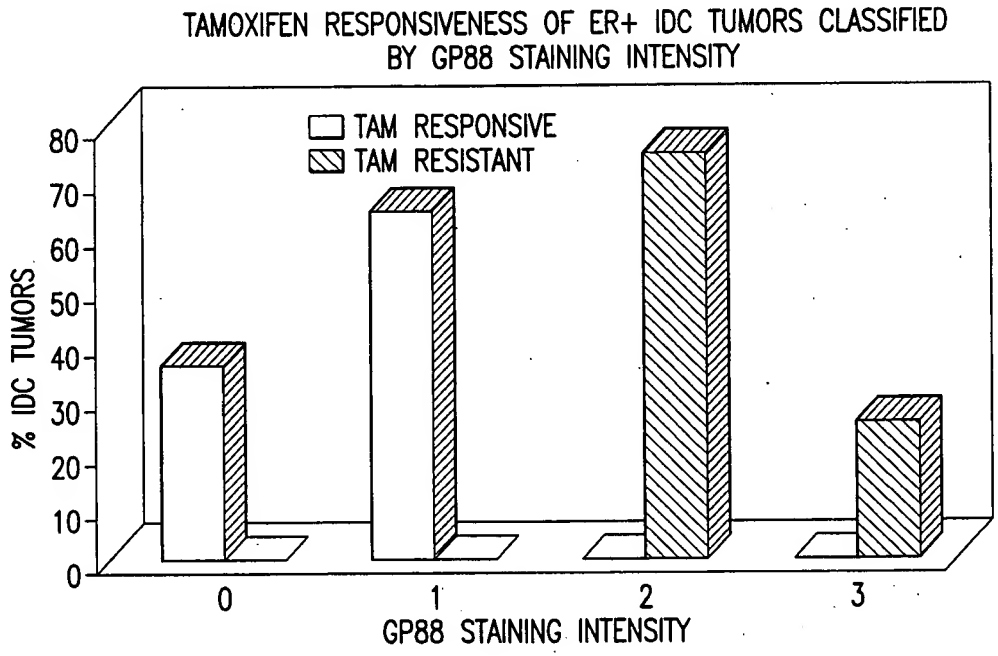
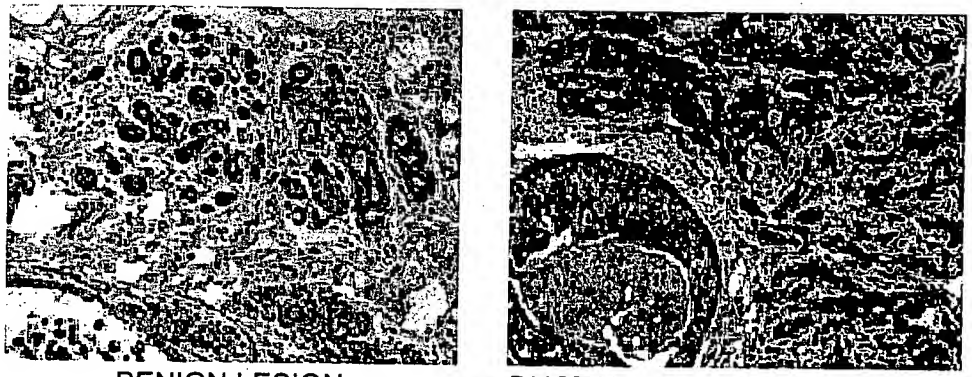


FIG.23



BENIGN LESION DUCTAL INVASIVE CARCINOMA

GP88 STAINING WITH ANTI-GP88 ANTIBODY
ON PARAFFIN EMBEDDED BREAST CANCER BIOPSIES
BY IMMUNOHISTOCHEMISTRY (IHC)

FIG.21

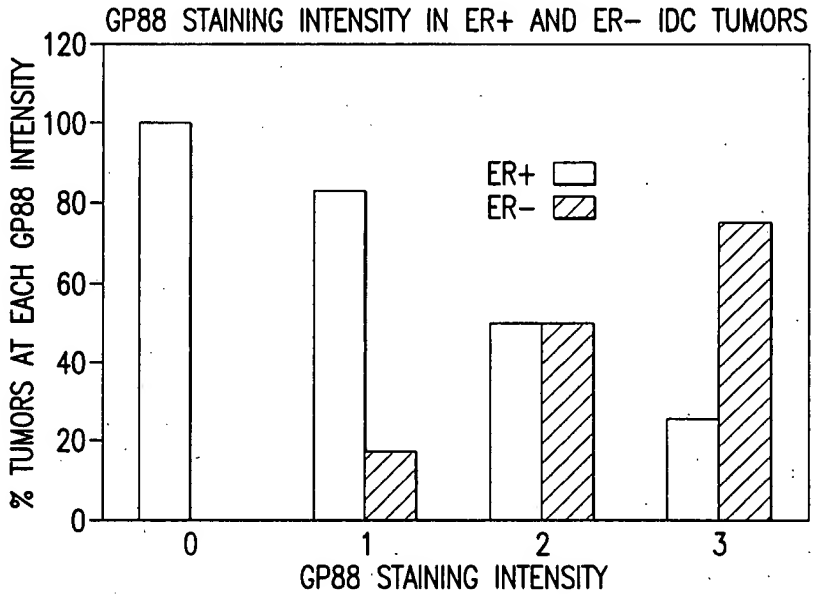


FIG.22